FORMULATION, EVALUATION OF SCAFFOLD AND COMPARATIVE STUDIES WITH TRANSFEROSOME FOR TOPICAL DELIVERY IN THE TREATMENT OF WOUND HEALING

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TABLE OF CONTENT

INTRODUCTION **3**

1.1 Scaffold 3

1.2 Topical drug delivery System 4

1.3 Transferosomes 4

1.4 Wound healing 5

2. REVIEW OF LITERATURE **6**

2.1 DRUG PROFILE 6

2.2 HENNA 6

2.2.5Pregnancy/Lactation 6

i. Interaction 6

2.2.6.1 Lithium interacts with HENNA 6

2.2.8.1 Insufficient Evidence for 7

2.2.9 Pharmacology action- 7

2.3.1.2 Description 8

2.3.1.4 Substance details 8

2.3.1.5 Physical properties 8

2.3.1.6 Chemical properties 8

2.3.1.7 Exposure 9

2.3.1.8 Entering the environment 9

2.3.1.9.1 Industry sources 9

2.3.1.9.2 Natural sources 9

2.3.1.9.3 Transport sources 9

2.3.1.9.4 Consumer products 9

3. RATIONALE OF THE STUDY **15**

4. AIM AND OBJECTIVE **16**

5. PLAN OF WORK **17**

6. MATERIAL & METHOD: **18**

7. EVALUATION PARAMETER **20**

CHAPTER-8 21

8. REFERENCES 21

CHAPTER-1

INTRODUCTION

1.1 Scaffold

Tissue engineering has grown in importance as a study area during the last 20 years, because it allows the functional recovery of organs and tissues which have undergone damage. The largest organ and the outermost layer of the human body is the skin. By developing structures that have structures and biological processes that really are similar to those of native tissues, Significant improvements in wound healing have been made possible through skin tissue engineering and regeneration [1]. In large part as a result of the advancement of 3D printing technology, new 3D bioprinting processes that can produce more detailed, practical, and customised three-dimensional constructs with superior imitation behaviour have been developed. In this context, it is essential to analyse the various 3D printing techniques and the associated printing parameters. 3D bioprinting of tissues is an additive manufacturing technique used to create biocompatible 3D constructions that resemble natural systems using a computer-generated design. In terms of automation, normalisation for therapeutic uses, and precision when it comes to the inclusion of living cells, growth factors, and other bimolecular compounds, 3D bioprinted dermal substitutes are superior to conventional methods for skin regeneration. The utilisation of bioinks, or bioprintable materials, is required for the development of complex three-dimensional matrices for skin engineering and wound healing via 3D bioprinting. Numerous natural polymer hydrogels, such as collagen, alginate, chitosan, hyaluronic acid, and cellulose, have been used to create bioinks [2]. Printed materials made of synthetic-based biopolymers have also been used to improve the dynamics of 3D constructions. These bioinks must possess a few key characteristics after printing, including good printability, mechanical stability, biocompatibility, biodegradability, non-toxicity, high availability, and high form fidelity, regardless of where they originate. Additionally, since they directly influence the immune response after the implantation of the printed scaffolds, the choice and availability of live cells are given top care while making the bioink. For this reason, when making skin bioprinting structures, it is preferred to co-culture primary skin cells (fibroblasts, keratinocytes, and melanocytes). The printed tissue or organ must continuously retain normal biological function, such as the capacity for cell migration and the rate of cell multiplication. In fact, the ability of bioinks to print as well as the ability of cells to disseminate, migrate, and proliferate are influenced by their

viscoelastic behaviour [3]. Since the term "tissue engineering" was first used in the late 1990s, biomaterial-based scaffolds have entered this field in an effort to provide structural stability and an environment that is favourable for cellular regeneration, thereby "imitating" native tissue in function. Applications for 3D scffolds span a wide range, including bone, nerve, muscle, tendon/ligament, regeneration, and many more.

The creation of these scaffolds has been researched using a variety of synthetic and natural polymers. The right choice of the material used to make tissue-engineered scaffolds is a vital step for the scaffold's effectiveness [4].

The use of 3D printing technology enables the production of specialised 3D structures with high resolution and controlled inner micro-architecture. There are numerous 3D printing techniques that can be used to produce scaffolds for tissue regeneration, each with advantages and disadvantages. 3D bioprinting is a manufacturing technique that can be used to produce artificial scaffolds or tissue structures by layer-by-layer deposition. Regarding tissue engineering applications, the two basic kinds of 3D bioprinting techniques are those that cannot directly print living cells into the structure and those that can [4]. Among the earliest are low-temperature deposition manufacturing (LTDM), stereolithography (SLA), selective laser sintering (SLS), and fused deposition modelling (FDM).

On the other hand, cellular bioprinting techniques use live cell-infused bioinks to produce the construct. These four bioprinting techniques can be broken down into four subcategories: laser, droplet, extrusion, and stereolithography [5].

1.2 Topical drug delivery System

An approach to giving medication that is applied to a particular area of the body, typically the skin, to treat various ailments is known as a topical drug delivery system. The typical forms of topical treatments include creams, ointments, patches, gels, lotions, and powders.

1.3 Transferosomes

In the early 1990s, transfersomes—a type of elastic or flexible vesicle—were first seen. An edge activator is included into the lipid bilayer structure to provide elasticity. These vesicles were first made using soybean phosphatidyl choline, sodium cholate, and a very little amount of ethanol. Transfersomes are applied to the skin in a non-occluded manner, and it has been demonstrated that they pass through the lipid lamellar areas of the stratum corneum as a consequence of the moisture or osmotic force in the skin. They

have been utilised both in vitro and in vivo as medication carriers for a variety of small compounds, peptides, proteins, and vaccines.

1.4 Wound healing

A biological system's very complicated physiological reaction to a thermal, chemical, mechanical, or physical insult is wound healing. A cascade of cells, matrix components, and other biological processes must cooperate to hasten healing and restore tissue integrity. The stages of wound healing that overlap are homeostasis, inflammation, proliferation/granulation, and remodeling/maturation. The healing, however, does not progress past the inflammatory stage when the course of healing deviates from the regular one. Burns, infections, or even issues brought on by pathological diseases can all prevent the body from mending normally.

To aid in wound care, speed up the healing process, and protect the wounded area from additional external mechanical and microbial stress, the wound site must be covered with an appropriate wound dressing. The typical wound dressings—cotton, bandages, and gauzes—are dry and do not provide the necessary moisture for wound healing. Additionally, dressings frequently stick to the wound bed due to wound drainage, hurting patients when they are removed. In order to overcome these constraints, substantial research has been conducted to discover novel defences that promote tissue regeneration and wound healing [10–15].

2. REVIEW OF LITERATURE

2.1 **DRUG PROFILE**

2.2 HENNA

2.2.1 Scientific Name(s): Lawsonia inermis

2.2.2 Common Name(s): Hina, Mehndi

The powdered leaf of a certain plant is known as henna. Medicine is made with it.

Inflammatory bowel illness (ulcerative colitis), bed sores, and other disorders are treated with henna.

2.2.3 Chemical compound structure

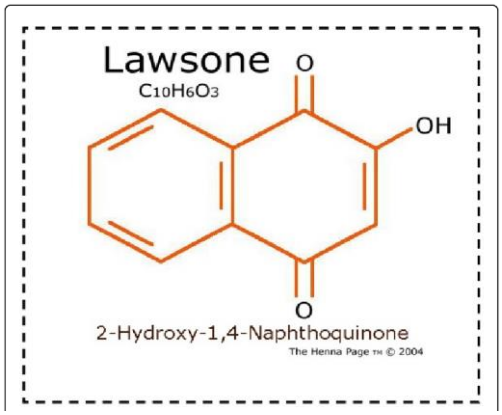


Fig no-1

2.2.4 Dosing

The right amount of henna to apply depends on a number of variables, including the user's age, health, and other circumstances. A suitable range of henna dosages cannot yet be determined due to a lack of data.

2.2.5Pregnancy/Lactation

The results indicated that the use of *Lawsonia inermis* during pregnancy may cause abortion.

i. Interaction

2.2.6.1 Lithium interacts with HENNA

Henna may have a similar effect to a "diuretic" or water pill. Henna use might impair the body's ability to eliminate lithium. Lithium levels may rise as a result, which may have negative side effects. You might need to adjust your lithium dosage.

2.2.7 Precaution-

When taken by mouth: Henna is **UNSAFE** when consumed orally. If you accidentally ingest henna, call your doctor right away. It may result in mortality, stomach distress, muscular wasting, renal failure, hemolytic anaemia, and the loss of red blood cells.

2.2.8 Use-

2.2.8.1 Insufficient Evidence for

- A skin response that is unfavourable and brought on by cancer medication (chemotherapy-induced acral erythema). Henna treatment may reduce chemotherapy-induced acral erythema in patients using the cancer medication capecitabine, according to preliminary studies.
- Hand- and foot-related nerve injury after cancer medication therapy. Henna powder application to the hands and feet appears to lessen the signs of nerve damage brought on by the cancer medication oxaliplatin in adult females.
- Skin repercussions brought on by coming into touch with a chemical (contact dermatitis). According to preliminary studies, patients with contact dermatitis from prosthetic legs may find relief from their discomfort and itching by using henna powder. But the redness can get worse.
- Bedbugs (pressure ulcers). According to preliminary studies, henna powder application to bed sores once accelerates healing when compared to olive oil or no therapy.
- Healing of wounds. Early studies in people who needed a surgical cut made at the entrance of their vagina after delivery suggest that putting henna 2% ointment to the surgical site for two weeks could somewhat lessen discomfort and redness. More evidence is needed to rate the effectiveness of henna for these uses.

2.2.9 Pharmacology action-

Lawsone, coumarine, anti-microbial, anti-tumorigenic, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, and anti-lipidemic are some of the principal pharmacological effects of henna and the chemicals extracted therefrom.

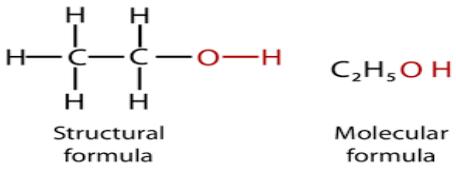
2.3 EXCIPIENT PROFILE

2.3.1 ETHANOL

2.3.1.2 Description

When diluted, ethanol can be found in beer, wine, and spirits. It is a topically applied component used in cosmetics, scents, and pharmaceutical preparations (such rubbing compounds, lotions, tonics, and colognes) to prevent skin infections. Fuels labelled as "ethanol mixed fuels" may contain ethanol, which is a solvent used in industry to dissolve hydrocarbons, waxes, resins, and other fats, oils, and fat-soluble substances. It is used to create many different chemical substances, such as lacquers, plastics and plasticizers, rubber and rubber accelerators, aerosols, mouthwash products, soaps, and cleaning solutions, polishes, surface coatings, dyes, inks, adhesives, preservatives, pesticides, explosives, petrol additives/substitutes, elastomers, antifreeze, yeast growth medium, human and veterinary medicines, and dehydrating agents**.**

2.2.1.3 Chemical Structure



2.3.1.4 Substance details

Substance name: Ethanol, Synonyms: ethyl alcohol, Molecular formula: C_2H_6O

2.3.1.5 Physical properties

Ethanol is a clear, colourless liquid with a pleasant scent and the distinct flavour of burning. It is highly flammable. Ethanol is used to dissolve other chemical compounds because it mixes easily with water and a range of organic liquids. Ethanol is included as a volatile organic component in the National Pollutant Inventory. Which have Melting Point: -114°C,Boiling Point: 78.5°C,Specific gravity: 0.8.

2.3.1.6 Chemical properties

Ethanol rapidly absorbs water from the air. It mixes readily with most organic liquids.

2.3.1.7 Exposure

Alcohol-containing foods and drinks, a wide range of consumer items, as well as a wide range of enterprises that use or manufacture ethanol can all expose people to ethanol.

Low concentrations of ethanol can be found in the environment; it is a natural byproduct of plant fermentation.

2.3.1.8 Entering the environment

Air and water both carry ethanol. It may go quite a distance since it is volatile and soluble in water.

2.3.1.9.1 Industry sources

Ethanol is created as a result of the manufacturing of basic chemicals, beverages, food, baked products, iron, steel, plastic, autos and auto parts, as well as industrial gear and equipment.

2.3.1.9.2 Natural sources

Ethanol is created by a variety of microbiological processes (by bacteria and fungus), as well as perhaps certain plants.

2.3.1.9.3 Transport sources

When utilised as a gasoline additive or replacement, ethanol can emit fumes from moving vehicles. Another source of ethanol is the evaporation of gasoline from car engines and fuel tanks.

2.3.1.9.4 Consumer products

Ethanol is a chemical compound that is found in alcoholic beverages, a variety of personal care and cosmetics items, household cleaners and polishes, pharmaceuticals, pesticides, and disinfectants, paints and paint-related items (like thinners), particle board products, lubricating oils, household and industrial inks (including printed materials), and pet products.

2.3.1.10 Concentrated sulphuric acid

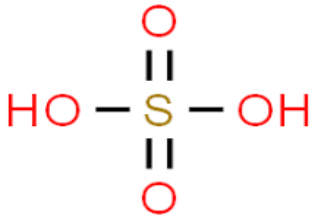
2.3.1.10.1 Discription

Four oxygen atoms, two hydrogen atoms, one sulphur atom, and sulfuric acid make up the chemical compound H2SO4. Depending on the concentration, sulfuric acid may be manufactured in a variety of forms and serves a variety of purposes.

2.3.1.10.2 Physical discription

A colourless, very corrosive oil, H2SO4 is. It is a dehydrating and oxidising agent. In addition to being used to produce explosives, paints, and fertilisers, sulfuric acid is also utilised to refine petroleum. A strong mineral acid that is soluble in water is sulfuric acid. An alchemist from the eighth century named Jabir ibn Hayyan is likely to have found the substance.

2.3.2.1.10.3 Chemical structure



Structure of sulphuric acid

2.3.1.10.4 Solubility

It is also known as "oil of vitriol." Strong mineral acid sulfuric acid, or H2S4, is soluble in water at all concentrations. Ore processing, fertiliser production, oil refining, wastewater treatment, and chemical synthesis are a few of its applications.

2.3.1.10.5 Uses-

is used to clean metals, get oil clear of contaminants, make chemicals like nitric acid and hydrochloric acid, make dye, medicines, detergents, and explosives, among other things.

- **Yuan TN., Zhang J. (2022)** delve into the topic of 3D bioprinting for spinal cord injuries As the science of mixing and printing cells and associated cytokines has steadily advanced, repair has been

the consequence. Complex biological scaffolds with precise cell localisation may be created using this method.

- **Gad S, Ayakar S (2021)** indicated that scaffolds of protein preparation are a technique for multi-enzyme assembly in biotechnology research, which demonstrated that the scaffolding enhances enzyme stability and catalytic efficiency and creates the ideal microenvironment for biochemical processes.

- **Afsharian YP , Rahimnejad M. (2021)** (Bioactive electrospun dressings that have produced successful acute and chronic wound therapy and tissue development are discussed in relation to bioactive electrospun scaffolds for wound healing applications. We also discuss the needs for electrospun dressings in order to develop a superior scaffold design.
- **Ahmad T, Grath MS. *et al.*, (2021)** demonstrated how to create wound healing scaffolds with precisely timed sequential therapeutic nano particle releases. Ultrasound was successfully used to successfully establish the feasibility of sequential release of various medicines within these scaffolds. These technologies offer a precise and adaptable way to administer therapeutic nanoparticles inside a verified regenerative template, and they can be used to give and research timed therapeutic delivery in tissue engineering applications such as wound healing..

- **Mourtas S. et al., (2021),** created multifunctional nanoliposomes with a possible use in the treatment of Alzheimer's disease contain a lipid derivative of curcumin and the ability to target the brain. The internalisation of MNPs by endothelial cells by atomic force microscopy suggests that trans-cellular trafficking may be a mechanism for the observed MNP crossing of the BBB.

- **Sivaraj S, Chen K. (2021)** discovered multifunctional nanoliposomes with a potential use in Alzheimer's disease that have a brain targeting activity and a lipid derivative of curcumin. MNPs were found to be internally processed by endothelial cells using atomic force microscopy, which raises the possibility that trans-cellular trafficking is the mechanism behind the observed MNP BBB crossing.

- **Gad S, Ayakar S (2021)** created multifunctional nanoliposomes with curcumin lipid derivative and brain targeting capabilities with potential uses for Alzheimer's disease. Atomic force microscopy revealed that endothelial cells ingested MNPs, pointing to trans-cellular trafficking as a potential mechanism for the observed MNP BBB crossing.

- **Kim D, Kim M. *et al*., (2022)** The use of conventional single-component bioinks for the printing of 3D in vitro liver constructs is still problematic due to the complex structural and physiological characteristics of the liver, as was shown in Multicomponent Hydrogel Bioinks Based on Natural Biomaterials for Bioprinting 3D Liver Tissues. This technique for bioprinting 3D functional in vitro liver tissue models has grown in popularity because of the many advantages of multicomponent bioinks, including improved mechanical qualities of the printed tissue construct and cell activity.
- **Babu, R.J; et al (2020),** Due to the decrease in gastro-intestinal ulcerations and other side effects, ease of distribution to legs and joints, and lowering of the dosage to diminish systemic toxicity of the drug, phenylbutazone (PBZ) transdermal administration may be useful in large animals. Deer skin was used to examine the formulations' in vitro skin-permeation kinetics using Franz diffusion cells. By increasing drug loading, MSA served as an effective skin-penetration enhancer in the liposomal gel of PBZ for deer.

- **Mehta M. *et al*., (2019)** explained Through the use of transferosomes, a non-invasive technology, transdermal drug delivery systems have been shown to be successful for topical and local pharmacological activity. Due to the multiple advantages of the transdermal drug delivery system and various permeation enhancers that would considerably increase the number of pharmaceuticals suitable for it, many researchers are interested in this technology.

- **Preeti *et al*., (2018)** With celecoxib transferosomal gel, rheumatoid arthritis is treated. The transferosomal gel for Celecoxib exhibited improved skin penetrating ability, better stability, higher entrapment efficiency, was simpler to scale up, and had the ability to boost one's own penetrating capacity, according to the results.
- **Crawford M. and Dagnino L. (2017)** explained The epidermal permeability barrier was created and maintained by scaffolding proteins. The outside-in epidermal permeability barrier in mammals is provided by the granular keratinocytes that have developed tight connections and are situated in the layers underneath the outermost cornified layer. Tight junctions are impermeable to

macromolecules, bacteria, water, ions, and water. They act as barriers on both the inside and the outside.

- **Honggeng G. *et al*., (2016**) developed Curcumin reduced the severity of acute Propioni bacterium acnes-induced by inhibiting HMGB1 expression in mice. Detected considerable hepatocyte apoptosis in pericentral areas. The use of curcumin significantly reduced hepatocyte apoptosis seven days after P. acnes priming.

- **Campione, E; *et al* (2020),** to assess the treatment of actinic erasotes correlating to keratotic and verrucous clinical variations for efficacy and tolerability of piroxicam 1% gel. After therapy, the AKESA score in these lesions was significantly decreased. Pruritus, slight erythema, dry skin, and seldom rash were side effects. According to our preliminary research, piroxicam has anti-tumorigenic properties and may be helpful in the chemoprevention of skin cancer.

- **Dubey et al (2019),** Ketolac, a transdermal gel formulation of ketorolac tromethamine, is used to alleviate nociceptive somatic discomfort. Ketorolac's skin penetration was further enhanced by the use of citric acid. According to in vitro diffusion studies, drug penetration significantly increases as drug concentration rises. The same did not, however, result in greater skin permeability during the in vivo investigation. While the effect on maximum serum concentration (Cmax) was negligible, the area under the concentration time curve (AUC (0-t0)) considerably increased with increasing dosage.
- **Berlin, J.M; *et al* (2018),** 64% of the participants in the active arm cleared 100 current target lesions, compared to 32% in the cryosurgery alone group (P.0001). Actinic Kerasotes are more common than before, thus it's crucial to think about and assess new therapy choices.
- **Ridhima et al., (2018),** created curcumin medication delivery method with multiparticulate gastro retentive properties. By keeping the gastro retentive drug delivery system in the stomach for an extended period of time, it is possible to increase the bioavailability and absorption of curcumin. Curcumin floating medication delivery device created using emulsion solvent diffusion technique. The improved formulations exhibit good buoyancy and curcumin release that is regulated in vitro.
- **Upmanyu et al., (2017)** created floating curcumin microspheres to provide an increased bioavailability and prolonged retention in the upper GIT. Solvent evaporation was used to create

the microspheres. Curcumin floating microspheres demonstrated great floatability, good buoyancy, and extended drug release in in vitro studies. The primary releasing mechanism was discovered to be diffusion.

- **Antezana PE, Municoy S, et al.,(2022)** Whilst at work, I evaluated the 3D bioprinted scaffolds for wound healing. In this article, current developments in skin tissue engineering using a unique approach called 3D bioprinting are succinctly described. Along with the existing methods for bioprinting, a summary of bioink formulations, quality, and attributes is covered.

CHAPTER-3

3. RATIONALE OF THE STUDY

Tissue engineering and regenerative medicine have been significantly impacted by developments in nanoparticle (NP) synthesis and the requirement for control over nanoscale systems.Due to lack of scientific evidences the present study is associated with formulation, evaluation of scaffold and comparative studies with transferosome for topical delivery in the treatment of wound healing. The development formulation would be economic affordable, accessible and patient compliance as well superimposed those pharmaceuticals that are the nation economy and consumer credentials.

Poorly solubility of drug

Poorly permeability of drug

CHAPTER-4

4. AIM AND OBJECTIVE

4.1 AIM

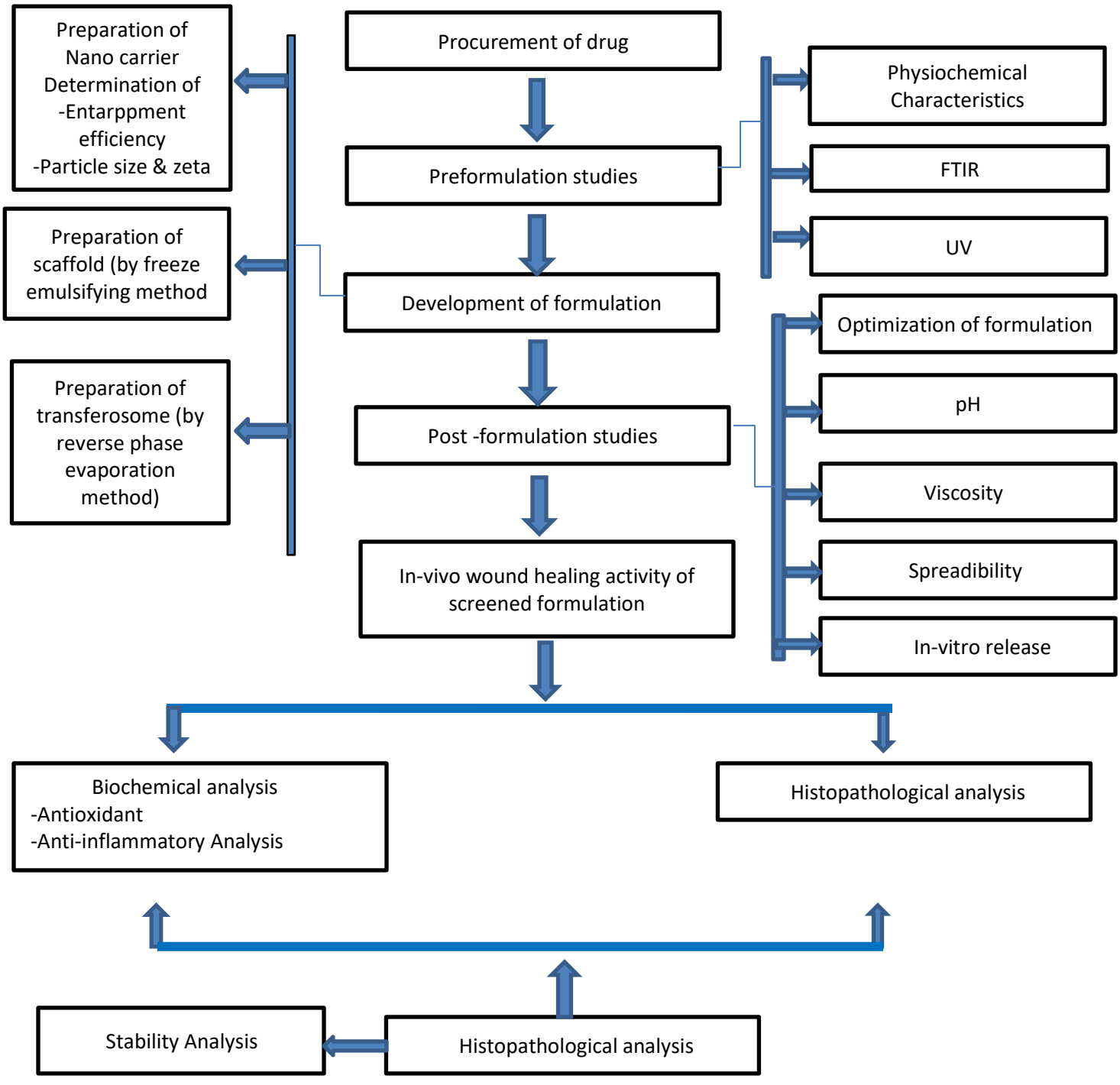
Formulation, evaluation of scaffold and comparative studies with transferosome for topical delivery in the treatment of wound healing.

4.2 OBJECTIVES

1. Assessment of pre-formulation studies
2. Development of formulation with various type of polymer.
3. Assessment of post formulation studies
4. Assessment of wound healing property of developed scaffold/transferosome using in-vivo model
5. Assessment of stability studies of best active formulations.

CHAPTER-5

5. PLAN OF WORK



CHAPTER-6

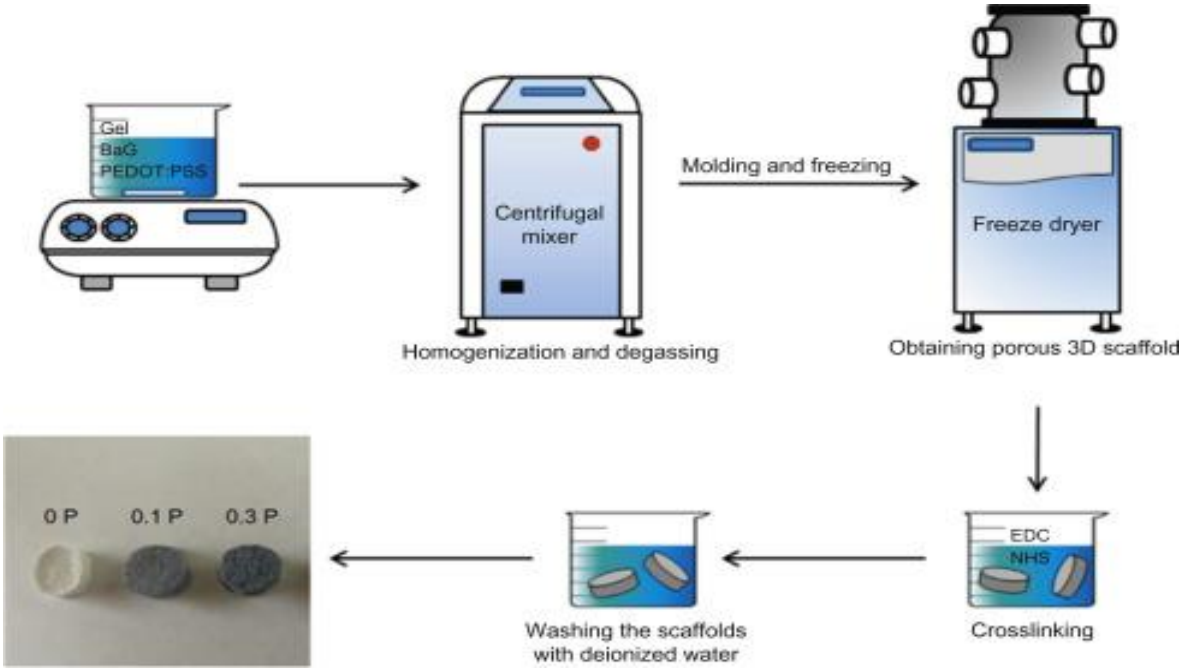
6. MATERIAL & METHOD:

6.1 Proposed List of chemical

6.2 METHOD OF PREPARATION

Freeze drying/emulsification (scaffold)

Freeze drying and emulsification are a promising method for scaffold preparation. The frozen water in polymer nanocomposites is instantly changed from a solid state to a gas state, without visible liquefaction, according to the sublimation process. The polymer is thoroughly dissolved in the appropriate solvent in the first stage. The water that is immiscible with this solution is then added to the mixture. As a result, the solvent and the water separate into phases. In order to achieve the solidification of solvent molecules, the contents are frozen. The final stage is lyophilizing under vacuum to remove the solvent and water. A scaffold for tissue engineering is made out of the very porous interconnecting polymeric structure that was thus obtained [18]. Although the final scaffold typically has a high porosity, it can be further tailored to meet specific needs by adjusting the freezing regime, water content, polymer concentration, the ice (solvent) crystal size, and the solution's pH. Freeze drying, which is frequently used to prepare scaffolds, has a number of problems, including a longer duration, high energy consumption, the use of cytotoxic solvents, and the creation of closed pores by gas foaming



Figno-2 Cell distribution analysis

In various studies pertaining to tissue engineering, the freeze drying approach has been employed successfully. [19–20].

Reverse phase evaporation (transferosome)

Transferosomes were created utilizing the reverse phase evaporation method, which calls for lipids and a surfactant to help create an ultra-deformable vesicle.

Accurately measured amounts of soya lecithin and cholesterol will add, follow by the addition of tween 80 as a surfactant. These ingredients will then dissolved in a solvent solution made up of a 3:1 ratio of diethyl ether and chloroform, and left at room temperature for 24 hours to form a thin film. Additional medication solution with span 80 and phosphate buffer added while being sonicated. Transferosomal suspension (DMSO) is added to each solution as a permeation enhancer.

CHAPTER-7

7. EVALUATION PARAMETER

- Optimization of formulation
- pH
- Viscosity
- Spreadibility
- In-vitro release

CHAPTER-8

8. REFERENCES

1. C.L. Kohnhorst, M. Kyoung, M. Jeon, D.L. Schmitt, E.L. Kennedy, J. Ramirez, S. An,Identification of a multi-enzyme complex for glucose metabolism in living cells, J. Biol. Chem. 292 (22) (2021) 9191–9203.
2. J. Kim, J.W. Grate, P. Wang, Nanobiocatalysis and its potential applications, Trends Biotechnol. 26 (11) (2018) 639–646
3. S. Asmat, A.H. Anwer, Q. Husain, Immobilization of lipase onto novel constructed polydopamine grafted multiwalled carbon nanotube impregnated with magnetic cobalt and its application in synthesis of fruit flavours, Int. J. Biol. Macromol. 140 (2019) 484–495.
4. C.M. Fung, J.S. Lloyd, S. Samavat, D. Deganello, K.S. Teng, Facile fabrication of electrochemical ZnO nanowire glucose biosensor using roll to roll printing technique, Sensors and Actuators, B: Chem. 247 (2017) 807–813.
5. Y. Wang, D. Chen, G. Wang, C. Zhao, Y. Ma, W. Yang, Immobilization of cellulase on styrene/maleic anhydride copolymer nanoparticles with improved stability against pH changes, Chem. Eng. J.l 336 (2018) 152–159.
6. B. Boscolo, F. Trotta, E. Ghibaudi, High catalytic performances of Pseudomonas fluorescens lipase adsorbed on a new type of cyclodextrin-based nanosponges, J. Mol. Catalysis B: Enzymatic 62 (2) (2010) 155–161.
7. P. Wu, F. Luo, Z. Lu, Z. Zhan, G. Zhang, Improving the catalytic performance of pectate lyase through pectate lyase/Cu3(PO4)2 hybrid nanoflowers as an immobilized enzyme, Front. Bioeng. Biotechnol. 8 (2020) 280
8. C. Lin, K. Xu, R. Zheng, Y. Zheng, Immobilization of amidase into a magnetic hierarchically porous metal-organic framework for efficient biocatalysis, Chem. Commun. 55 (40) (2019) 5697–5700.
9. Silva-Torres, O., Bojorquez-Vazquez, L., Simakov, A., & Vazquez-Duhalt, R. (2019). Enhanced laccase activity of biocatalytic hybrid copper hydroxide nanocages. Enzyme and microbial technology, 128, 59–66.
10. Uzunoglu, The use of CeO2-TiO2 nanocomposites as enzyme immobilization platforms in electrochemical sensors, J. Turkish Chem. Society, Section A: Chem. 4 (3) (2017) 855–868.

11. Saeki H, Furue M, Furukawa F, *et al*. Guidelines for management of atopic dermatitis. J Dermatol2019; (36) 563-577.
12. Akdis CA, Akdis M, Simon D, *et a*l. T cells and T cell-derivedcytokines as pathogenic factors in the nonallergic form ofatopic dermatitis. J Invest Dermatol 2018;(113)628-634.
13. Drake L, Prendergast M, Maher R, *et al*. The impact of tacrolimus ointment on health-related quality of life of adult and pediatric patients with atopic dermatitis. J Am AcadDermatol 2019; (44)65-72.
14. Wahlgren CF. Itch and atopic dermatitis: an overview.JDermatol 2021; (26)770-779.
15. Rubins A, Gutmane R, Valdmane N, et al. Pharmacokinetics of 0.1% tacrolimus ointment after first and repeated application to adults with moderate to severe atopic dermatitis. J Invest Dermatol 2021; (125) 68-71.
16. Beck LA. The efficacy and safety of tacrolimus ointment: a clinical review. J Am AcadDermatol 2020; (53) 165-170.
17. Sengoku T, Kishi S, Sakuma S, et al. FK506 inhibition of Histamine release and cytokine production by mast cells andbasophils. Int J Immunopharmacol 2018; (22)189-201.
18. Storeys GO, Comer M, Scott DL. Chronicarthritis before 1876: Early British cases suggesting rheumatoid arthritis. *Ann Rheum Dis.* 2017; (53): 557-560.
19. Pomahac B, Svensjo T, Yao F, Brown H, Eriksson E (2021) Tissue engineering of skin. Crit Rev Oral Biol Med 9: 333–344.
20. Choi W, Wolber R, Gerwat W, Mann T, Batzer J, et al. (2016) The fibroblastderived paracrine factor neuregulin-1 has a novel role in regulating the constitutive color and melanocyte function in human skin. J Cell Sci 123: 3102– 3111.
21. Boyce ST (2017) Design principles for composition and performance of cultured skin substitutes. Burns 27: 523–533.
22. Ma L, Gao C, Mao Z, Zhou J, Shen J, et al. (2018) Collagen/chitosan porous scaffolds with improved biostability for skin tissue engineering. Biomaterials 24: 4833–4841.
23. Schulz JT 3rd, Tompkins RG, Burke JF (2021) artificial skin. Annu Rev Med 51: 231–244.
24. Seet WT, Manira M, Khairul Anuar K, Chua KH, Ahmad Irfan AW et al. (2021) Shelf-Life Evaluation of Bilayered Human Skin Equivalent, MyDermTM. PLoS ONE

25. Tripathi M, Pandey M, Nepal B, Rai H, Bhattarai B (2022) Evaluation of lung infiltration score to predict postural hypoxemia in ventilated acute respiratory distress syndrome patients and the lateralization of skin pressure sore. Indian J Med Sci 63: 392–401.
26. Corin KA, Gibson LJ (2016) Cell contraction forces in scaffolds with varying pore size and cell density. Biomaterials 31: 4835–4845.
27. Duan X, Sheardown H (2015) Crosslinking of collagen with dendrimers. J Biomed Mater Res A 75: 510–518.
28. Schneider A, Garlick JA, Egles C (2018) Self-assembling peptide nanofiber scaffolds accelerate wound healing. PLoS One 1401-1410.
29. Friess W (1998) Collagen – biomaterial for drug delivery. Eur J Pharm Biopharm 45: 113–136.
30. Nimni ME, Cheung D, Strates B, Kodama M, Sheikh K (2016) Chemically modified collagen: a natural biomaterial for tissue replacement. J Biomed Mater Res 21: 741–771.
31. 18. Nam K, Kimura T, Funamoto S, Kishida A (2020) Preparation of a collagen/ polymer hybrid gel designed for tissue membranes. Part I: controlling the polymer-collagen cross-linking process using an ethanol/water co-solvent. Acta Biomater 6: 403–408.
32. Nam K, Kimura T, Funamoto S, Kishida A (2016) Preparation of a collagen/ polymer hybrid gel for tissue membranes. Part II: in vitro and in vivo biological properties of the collagen gels. Acta Biomater 6: 409–417.
33. Ruszczak Z (2015) Effect of collagen matrices on dermal wound healing. Adv Drug Deliv Rev 55: 1595–1611.
34. Clark RA, Ghosh K, Tonnesen MG (2016) Tissue engineering for cutaneous wounds. J Invest Dermatol 127: 1018–1029.
35. Ishibuchi H, Abe M, Yokoyama Y, Ishikawa O (2022) Induction of matrix metalloproteinase-1 by small interfering RNA targeting connective tissue growth factor in dermal fibroblasts from patients with systemic sclerosis. Exp Dermatol 19: e111–116